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INVESTOR IN PEOPLE

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NP10 8QQ

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Dated 31 March 2003

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Pate Form 1/77

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The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference — GMPL/PMS/P33066

2. Patent application number *(The Patent Office will fill in his pa)* — 0213646.3

8202293 001
13 JUN 2002

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Glaxo Group Limited
Glaxo Wellcome House, Berkeley Avenue,
Greenford, Middlesex UB6 0NN, Great Britain

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation — United Kingdom

see continuation sheet for further applicant(s) →

4. Title of the invention — Novel Compounds

5. Name of your agent *(if you have one)*

Corporate Intellectual Property

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

GlaxoSmithKline
Corporate Intellectual Property CN925.1
980 Great West Road
BRENTFORD
Middlesex TW8 9GS

Patents ADP number *(if you know it)*

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer yes if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is named as an applicant, or
c) any named applicant is a corporate body
See note (d)

Patents Form 1/77

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Continuation sheets of this form	1
Description	14
Claim(s)	0
Abstract	0
Drawings	0

10. If you are also filing any of the following, state how many against each item.

Priority Documents

Translations of priority documents

Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*

Request for preliminary examination and search *(Patents Form 9/77)*

Request for substantive examination *(Patents Form 10/77)*

Any other documents *(please specify)*

11. We request the grant of a patent on the basis of this application

Signature S C Hockley Date 12-Jun-02

12. Name and daytime telephone number of person to contact in the United Kingdom: S C Hockley 01279 644355

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CONTINUATION SHEET

Reference: GMPL/PMS/P33066

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Novel Compounds

This invention relates to newly identified gemini surfactant compounds having a cyclic bridge structure linking the two headgroups, to the use of such compounds and to their production. The invention also relates to the use of the cyclic bridge gemini compounds to facilitate the transfer of compounds into cells for drug delivery.

Surfactants are substances that markedly affect the surface properties of a liquid, even at low concentrations. For example surfactants will significantly reduce surface tension when dissolved in water or aqueous solutions and will reduce interfacial tension between two liquids or a liquid and a solid. This property of surfactant molecules has been widely exploited in industry, particularly in the detergent and oil industries. In the 1970s a new class of surfactant molecule was reported, characterised by two hydrophobic chains with polar heads which are linked by a hydrophobic bridge (Deinega,Y *et al.*, *Kolloidn. Zh.* **36**, 649, 1974). These molecules, which have been termed "gemini" (Menger, FM and Littau,CA, *J.Am.Chem.Soc.* **113**, 1451, 1991), have very desirable properties over their monomeric equivalents. For example they are highly effective in reducing interfacial tension between oil and water based liquids and have a very low critical micelle concentration (Menger, FM and Keiper, JS, *Angewandte. Chem. Int. Ed. Engl.*, 2000, **39**, 1906).

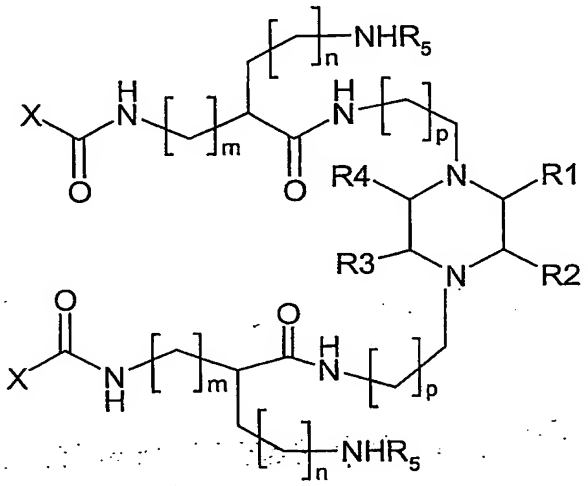
Cationic surfactants have been used *inter alia* for the transfection of polynucleotides into cells in culture, and there are examples of such agents available commercially to scientists involved in genetic technologies (for example the reagent Tfx™-50 for the transfection of eukaryotic cells available from Promega Corp. WI, USA).

The efficient delivery of DNA to cells *in vivo*, either for gene therapy or for antisense therapy, has been a major goal for some years. Much attention has concentrated on the use of viruses as delivery vehicles, for example adenoviruses for epithelial cells in the respiratory tract with a view to corrective gene therapy for cystic fibrosis (CF). However, despite some evidence of successful gene transfer in CF patients, the adenovirus route remains problematic due to inflammatory side-effects and limited transient expression of the transferred gene. Several alternative methods for *in vivo* gene delivery have been investigated, including studies using cationic surfactants. Gao,X *et al. Gene Ther.* **2**, 710-722,1995 demonstrated the feasibility of this approach with a normal human gene for CF transmembrane conductance regulator (CFTR) into the respiratory epithelium of CF mice using amine carrying cationic lipids. This group followed up with a liposomal CF gene therapy trial which, although only partially successful, demonstrated the potential for this approach in humans (Caplen, NJ. *et al.*,

Nature Medicine, 1, 39-46, 1995). More recently other groups have investigated the potential of other cationic lipids for gene delivery (Miller, A, *Angew. Int. Ed. Engl.*, 37, 1768-1785, 1998), for example cholesterol derivatives (Oudrhiri,N et al. *Proc.Natl.Acad.Sci.* 94, 1651-1656, 1997). This limited study demonstrated the ability of these cholesterol based compounds to facilitate the transfer of genes into epithelial cells both *in vitro* and *in vivo*, thereby lending support to the validity of this general approach.

These studies, and others, show that in this new field of research there is a continuing need to develop novel low-toxicity surfactant molecules to facilitate the effective transfer of polynucleotides into cells both *in vitro* for transfection in cell-based experimentation and *in vivo* for gene therapy and antisense treatments. Gemini surfactants based on cysteine (Camilleri, P. *and al.*, patent WO9929712) or on spermine or diamine (Camilleri, P. *and al.*, patent WO0076954) have already been synthesised probing the usefulness of this approach. The present invention seeks to overcome the difficulties exhibited by existing compounds.

The invention relates to cyclic bridge gemini compounds having a general structure of formula (I):

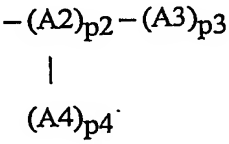


(I)

where R_1 to R_4, which can be the same or different, are selected from H, O or C_qH_{2q+1}, where q = 0 to 6 with the proviso that when R_1 and R_3 are O, or where R_1 and R_2 are O then R_2 and R_4 or R_3 and R_4, respectively, are H;

m = 0 to 6, n = 0 to 6, p = 0 to 5

and where R_5 is either H or a peptide group formed from one or more amino acids linked together by amide (CONH) bonds and further linked to the gemini backbone by amide bonds, in a linear or branched manner, having the general formula (II):



(II)

where the values for p1 and p2, which may be the same or different, are from 0 to 5, preferably 1; and the values for p3 and p4, which may be the same or different, are from 0 to 5, preferably 0;
A1, A3 and A4, which may be the same or different, is an amino acid selected from serine, lysine, ornithine, threonine, histidine, cysteine, arginine, tyrosine, 2,4-diaminobutyric acid and 2,3-diaminopropionic acid; and
A2 is an amino acid selected from lysine, ornithine, histidine, 2,4-diaminobutyric acid and 2,3-diaminopropionic acid;
and X is a saturated or unsaturated hydrocarbon chain having up to 24 carbon atoms and linked to the gemini backbone by an amide bond;
or
a salt, preferably a pharmaceutically acceptable salt thereof.

In a preferred embodiment m = 0, n = 3 and p = 1. In a further preferred embodiment R_5 = H.

Preferably the hydrocarbon chain, X, is selected from:

$(CH_2)_{10}CH_3$
$(CH_2)_{12}CH_3$
$(CH_2)_{14}CH_3$
$(CH_2)_{16}CH_3$
$(CH_2)_{18}CH_3$
$(CH_2)_{20}CH_3$
$(CH_2)_7$ CH=CH$(CH_2)_5CH_3$ natural mixture
$(CH_2)_7$ CH=CH$(CH_2)_7CH_3$ natural mixture
$(CH_2)_7$ CH=CH$(CH_2)_5CH_3$ Cis
$(CH_2)_7$ CH=CH$(CH_2)_7CH_3$ Cis
$(CH_2)_7$ CH=CH$(CH_2)_5CH_3$ Trans

$(CH_2)_7$ CH=CH$(CH_2)_7CH_3$ Trans

$(CH_2)_7$ CH=CHCH_2CH=CH$(CH_2)_4CH_3$

$(CH_2)_7$ (CH=CH$CH_2)_3CH_3$

$(CH_2)_3$ CH=CH$(CH_2CH=CH)_3(CH_2)_4CH_3$

$(CH_2)_7$ CHCH$(CH_2)_7CH_3$

CHCHOH$(CH_2)_2CH_3$

Most preferably the hydrocarbon chain X is selected from $(CH_2)_7$ CH=CH$(CH_2)_7CH_3$ natural mixture, $(CH_2)_7$ CH=CH$(CH_2)_7CH_3$ Cis and $(CH_2)_7$ CH=CH$(CH_2)_7CH_3$ Trans.

Compounds of the present invention may be prepared from readily available starting materials using synthetic peptide chemistry well known to the skilled person for example as illustrated in the examples hereinbelow. The method used to prepare each compound in the examples is only illustrative. The skilled person will appreciate that other methods are suitable for the synthesis of these compounds using techniques well known in the art.

Another aspect of the invention relates to methods for using the cyclic bridge gemini compounds. Such uses include facilitating the transfer of oligonucleotides and polynucleotides into cells for antisense, gene therapy and genetic immunisation (for the generation of antibodies) in whole organisms. Other uses include employing the compounds of the invention to facilitate the transfection of polynucleotides into cells in culture when such transfer is required, in, for example, gene expression studies and antisense control experiments among others. The polynucleotides can be mixed with the compounds, added to the cells and incubated to allow polynucleotide uptake. After further incubation the cells can be assayed for the phenotypic trait afforded by the transfected DNA, or the levels of mRNA expressed from said DNA can be determined by Northern blotting or by using PCR-based quantitation methods for example the Taqman® method (Perkin Elmer, Connecticut, USA). Compounds of the invention offer a significant improvement, typically between 3 and 6 fold in the efficiency of cellular uptake of DNA in cells in culture, compared with compounds in the previous art. In the transfection protocol, the gemini compound may be used in combination with one or more supplements to increase the efficiency of transfection. Such supplements may be selected from, for example:

(i) a neutral carrier, for example dioleyl phosphatidylethanolamine (DOPE) (Farhood, H., *et al* (1985) *Biochim. Biophys. Acta,* 1235-1289);

(ii) a complexing reagent, for example the commercially available PLUS reagent (Life Technologies Inc. Maryland, USA) or peptides, such as polylysine or polyornithine peptides or peptides

comprising primarily, but not exclusively, basic amino acids such as lysine, ornithine and/or arginine. The list above is not intended to be exhaustive and other supplements that increase the efficiency of transfection are taken to fall within the scope of the invention.

In still another aspect, the invention relates to the transfer of genetic material in gene therapy using the compounds of the invention.

Yet another aspect of the invention relates to methods to effect the delivery of non-nucleotide based drug compounds into cells *in vitro* and *in vivo* using the compounds of the invention.

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"Amino acid" refers to dipolar ions (zwitterions) of the form $^{+}H_3NCH®CO_2^{-}$. They are differentiated by the nature of the group R, and when R is different from hydrogen can also be asymmetric, forming D and L families. There are 20 naturally occurring amino acids where the R group can be, for example, non-polar (e.g. alanine, leucine, phenylalanine) or polar (e.g. glutamic acid, histidine, arginine and lysine). In the case of un-natural amino acids R can be any other group which is not found in the amino acids found in nature.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNA's or RNA's containing one or more modified bases and DNA's or RNA's with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications have been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Transfection" refers to the introduction of polynucleotides into cells in culture using methods involving the modification of the cell membrane either by chemical or physical means. Such methods are described in, for example, Sambrook et al., *MOLECULAR CLONING: A*

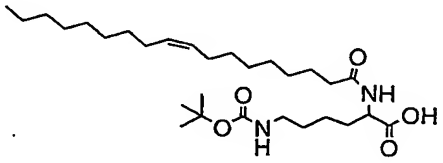
LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). The polynucleotides may be linear or circular, single-stranded or double-stranded and may include elements controlling replication of the polynucleotide or expression of homologous or heterologous genes which may comprise part of the polynucleotide.

The invention will now be described by way of the following examples.

EXAMPLES

Example 1

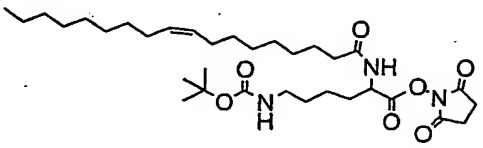
RG 00/781



To a solution of *N*-ε-(*tert*butoxycarbonyl)-L-lysine (1.24 g, 5.03 mmol) in THF (140 mL) were added successively a solution of K_2CO_3 (0.75 g, 5.43 mmol, 1.08 eq.) in water (20 mL) and oleoyl succinimidate (1.92 g, 5.06 mmol, 1 eq.). The reaction was stirred at RT for 20 h and most of THF was evaporated. Water and $CHCl_3$ (30 mL each) were added and the organic layerwas separated. The aqueous layer was acidified to pH 2 and extracted twice with $CHCl_3$ (2 x 30 mL). The organic layer was washed with water and brine (20 mL each), dried (Na_2SO_4), filtered and evaporated to give an oil. Yield : 2.46 g (4.82 mmol, **96 %**). ^1H NMR (400 MHz, d_6-DMSO): δ 12.4 (m, 1 H^{OH}), 7.92 (d, 1 H, $J = 7.8$, HN^{α}), 6.70 (t, 1 H, $J = 6.0$, HN^{ε}), 5.29 (m, 2 $CH^{9,10}$), 4.10 (dt, 1 H, J = 5.0, 8.9, CH^{α}), 2.85 (q, 2 H, $J = 6.2$, CH_2^{ε}), 2.07 (dt, 2 H, $J = 2.2$, 7.0, CH_2^{2}), 1.95 (q, 4 H, J = 6.0, $CH_2^{8,11}$), 1.62 (m, 1 H, CH^{β}), 1.51 (m, 1 H, CH^{β}), 1.45 (m, 2 H, CH_2^{3}), 1.33 (s, 9 H, $C(CH_3)_3$), 1.2 (m, 26 H, 2 $CH_2^{\gamma,\delta}$ and 10 CH_2 oleoyl), 0.82 (t, $J = 6.4$, 3 H, CH_3^{18}).

Example 2

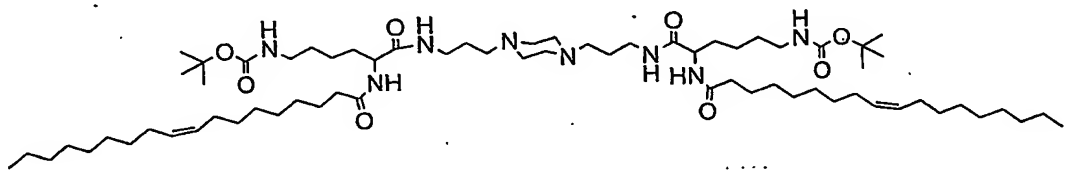
RG 00/366



To a solution of *N*-α-oleoyl-*N*-ε-(*tert*-butyloxycarbonyl)-L-Lysine (1.80 g, 3.52 mmol) in THF (80 mL) were added successively *N*-hydroxysuccinimide (0.41 g, 3.56 mmol, 1.01 eq.) and DCC (0.73 g, 3.54 mmol, 1.01 eq.). The reaction was stirred for 16 h at RT. The precipitate was filtered and washed with EtOAc (30 mL). The filtrate was concentrated and redissolved in EtOAc and filtered again. The residue was dissolved in $CHCl_3$ and precipitated with Et_2O to give N-α-oleate-N-ε-(*tert*-butyloxycarbonyl)-L-Lysinyl succinimidate as a white solid. Yield : 1.98 g (93 %). NMR ^1H (400 MHz, $CDCl_3$) : δ 6.11 (m, 1 H, HN^{α}), 5.38 (m, 2 H, $H^{9,10}$), 4.94 (m, 1 H, CH^{α}), 4.65 (m, 1 H, HN^{ε}), 3.12 (m, 2 H, CH_2^{ε}), 2.79 (s, 4 H, 2 CH_2^{Su}), 2.20 (t, J = 6.1, 2 H, CH_2^{2}), 2.00 (m, 5 H, CH^{β} and 2 $CH_2^{8,11}$), 1.84 (m, 1 H, CH^{β}), 1.63 (m, 2 H, CH_2^{3}), 1.48 (m, 4 H, 2 $CH_2^{\gamma,\delta}$), 1.37 (s, 9 H, 3 CH_3), 1.27 (m, 20 H, 10 CH_2 oleoyl), 0.83 (t, J = 6.3 Hz, 3 H, CH_3^{18}).

Example 3

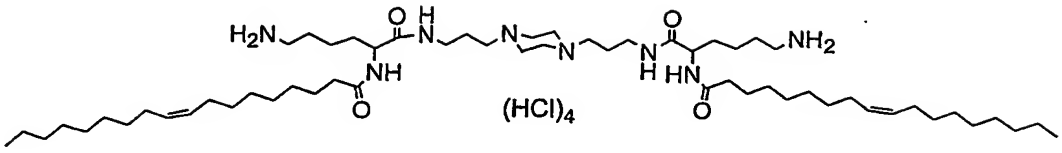
RG 00/518



To a solution of activated aminoacid (610 g, 1.0 mmol) in THF (45 mL) was added bis-*N*-aminopropyl-piperazine (0.081 mL, 0.5 mmol, 0.5 eq.) and then potassium carbonate (0.15 g, 1.1 mmol, 2.2 eq.) in water (10 mL) and the reaction was stirred at RT for 20 h. Most of the THF was removed under vacuum, $CHCl_3$ was added and the organic layer was extracted, washed with water (20 mL), dried (Na_2SO_4), filtered and evaporated. The residue was purified by column chromatography on silica ($CHCl_3$ / MeOH : 8.5 / 1.5, Rf = 0.3) to give a white solid. Yield : 490 mg (0.413 mmol, 83 %). ^1H NMR (400 MHz, $CDCl_3$): δ 7.68 (m, 2 H, 2 NHC^{1}), 6.46 (m, 2 H, 2 $N^{\alpha}H$), 5.32 (m, 4 H, 2 $CH^{9,10}$), 4.86 (m, 2 H, 2 N^{ε}Hboc), 4.33 (q, 2 H, J = , 2 CH^{α}), 3.38 (m, 2 H, $CH^{1'}$), 3.28 (m, 2 H, $CH^{1'}$), 3.05 (m, 4 H, 2 CH_2^{ε}), 2.47 (m, 12 H, 2 $CH_2^{3'}$ and 4 $CH_2^{2'}$), 2.18 (t, 4 H, J = , 2 CH_2^{2}), 1.99 (m, 8 H, 2 $CH_2^{8,10}$), 1.82 – 1.54 (m, 12 H, 2 $CH_2^{2'}$, 2 CH_2^{3} and 2 CH_2^{β}), 1.48 (m, 4 H, 2 CH_2^{γ}), 1.42 (s, 18 H, 2 $(CH_3)_3$), 1.21 (m, 24 H, 10 CH_2^{Ol} and 2 CH_2^{γ}), 0.87 (t, 6 H, J = 6.4, 2 CH_3^{18}). ^{13}C NMR (400 MHz, CD_3OD): δ 175.2, 173.4, 157.5, 129.9, 129.8, 78.8, 56.0, 53.8, 52.9, 41.3, 40.1, 37.7, 35.9, 32.1, 31.9, 29.9, 29.6, 29.5, 29.4, 29.3, 27.9, 27.2, 26.2, 26.0, 23.3, 22.8, 13.5.

Example 4

RG 00/522 = GSC 170

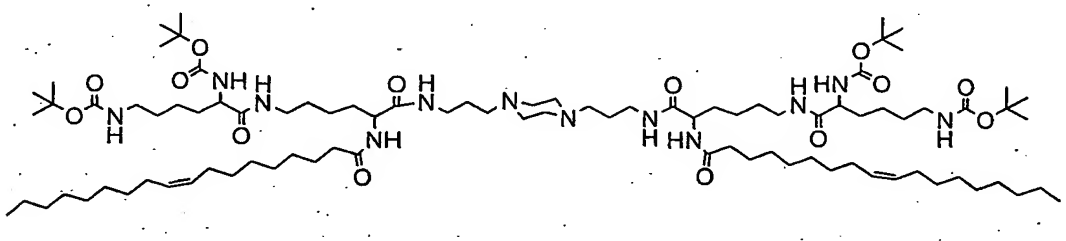


To a solution of protected RG00/518 (490 mg, 0.413 mmol) in MeOH (10 mL) was added concentrated HCl (10 mL). The reaction was stirred for 1 h and the solvent was then evaporated. The residue was redissolved in water (40 mL), filtered and evaporated. In this case it was impossible to precipitate the compound using MeOH / Et_2O. A white solid was collected. Yield : 381 mg (0.337 mmol, **81 %**). HRMS (+ES) : 985.8879 calculated for $C_{58}H_{113}N_8O_4$, found 985.8890.

Note : a similar procedure using TFA and neutralisation with K_2CO_3 was used to isolate the free amine in a quantitative yield. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.78 (2 d, 4 H, J = 8.0, 4 NHCO), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.12 (q, 2 H, J = 6.2, 2 CH^{α}), 3.04 (m, 4 H, 2 $CH_2^{1'}$), 2.47 (m, 8 H, 4 CH_2^{4}), 2.29 (m, 4 H, 2 NH_2), 2.19 (t, 4 H, J = 6.2, 2 $CH_2^{3'}$), 2.05 (m, 4 H, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.35 – 1.69 (m, 12 H, 2 $CH_2^{2'}$, 2CH_2^{3} and 2 CH_2^{β}), 1.21 (m, 26 H, 10 CH_2^{OI} and CH_2^{δ} and CH_2^{γ}), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 5

RG 00/794

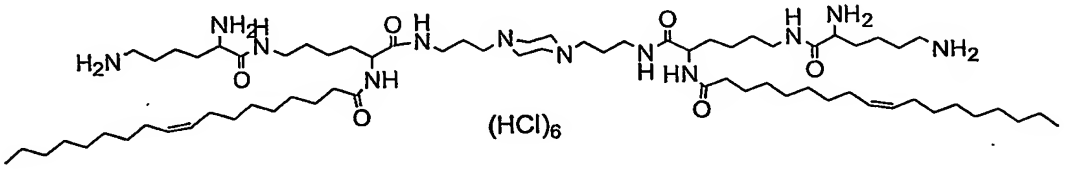


To a solution of bis aminocompound (150 mg, 0.152 mmol) in THF (40 mL) was added successively a solution of K_2CO_3 (42 mg, mmol, 2.1 eq.) in water (2 mL) and *N,N*-bis-(tertbutoxycarbonyl)-L-lysinyl succinimidate (140 mg, 0.304 mmol, 2.0 eq.) in THF (10 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer extracted, washed with water (2 x 10 mL) and brine (20 mL). After

drying (Na_2SO_4), filtration and evaporation, the residue is purified on SiO_2 (eluent : $CHCl_3$ / MeOH / NH_4OH: 87 / 12 / 1, Rf = 0.28). Et_2O is then added and the resulting white solid filtered off. Yield : 0.124 g (0.076 mmol, **50 %**). ^1H NMR (400 MHz, d^6-DMSO) : δ 7.75 (m, 4 H, 2 $NH^{\alpha 1}$ and 2 NHC^{1}), 7.68 (t, 2 H, *J* = , 2 $NH^{\varepsilon 1}$), 6.69 (t, 2 H, J = , 2 $NH^{\varepsilon 2}$), 6.63 (d, 2 H, *J* = , 2 $NH^{\alpha 2}$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.10 (q, 2 H, *J* = , 2 $CH^{\alpha 1}$), 3.78 (q, 2 H, *J* = , 2 $CH^{\alpha 2}$), 3.00 (m, 6 H, 2 $CH_2^{\varepsilon 1}$ and 2 $CH^{1'}$), 2.95 (m, 2 H, 2 $CH^{1'}$), 2.84 (m, 4 H, 2 $CH_2^{\varepsilon 2}$), 2.29 (m, 8 H, 4 $CH_2^{4'}$), 2.19 (m, 4 H, 2 $CH_2^{3'}$), 2.06 (t, 4 H, *J* = , 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.55 – 1.4 (m, 16 H), 1.32 (s, 36 H, 4 $C(CH_3)_3$), 1.20 (m, 48 H), 0.82 (t, 6 H, *J* = 6.4, 2 CH_3^{18}).

Example 6

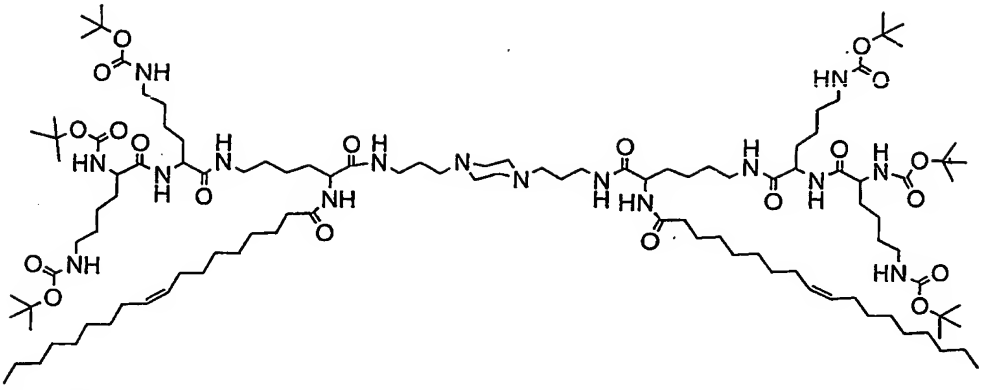
RG00/813 = GSC 184



To a solution of RG00/794 (124 mg, 0.0755 mmol) in MeOH (5 mL) was added concentrated HCl (5 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vacuum. The residue was dissolved in water, filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected. Yield : 0.102 g (0.070 mmol, **93 %**). ^1H NMR (400 MHz, d_6-DMSO) : δ 8.66 (d, 2 H, *J* = 7.8, 2 $NH^{\varepsilon 1}$), 8.28 (m, 6 H, 2 $N^{\alpha}H_3^+$), 8.09 (m, 2 H, 2 NHC^{1}), 8.05 (m, 6 H, 2 $N^{\varepsilon}H_3^+$), 7.98 (d, 2 H, *J* = 7.0, 2 $N^{\alpha}H$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.09 (m, 2 H, 2 $CH^{\alpha 1}$), 3.72 (m, 2 H, 2 $CH^{\alpha 2}$), 3.65 (m, 2 H, 2 NH^+), 3.10 (m, 12 H, 2 $CH_2^{\varepsilon 1}$, 2 $CH_2^{3'}$ and 2 $CH_2^{1'}$), 2.74 (m, 8 H, 2 $CH_2^{\varepsilon 2}$), 2.11 (t, 4 H, *J* = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.82 (m, 2 H, 2 $CH_2^{\delta 1}$), 1.70 (m, 2 H, 2 $CH_2^{\beta 2}$), 1.57 (m, 6 H, 2 $CH_2^{\delta 2}$ and 2 $CH^{\beta 1}$), 1.50 – 1.15 (m, 66 H), 0.84 (t, 6 H, *J* = 6.4, 2 CH_3^{18}). MS (+ES) : 1264.9 [M+Na].

Example 7

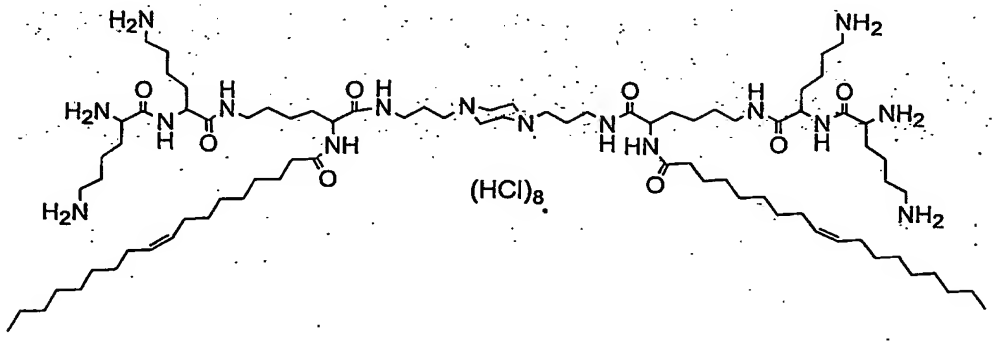
RG 00/793



To a solution of bis aminocompound (146 mg, 0.148 mmol) in THF (40 mL) was added successively a solution of K_2CO_3 (40 mg, mmol, 2.1 eq.) in water (2 mL) and *N,N*-bis-(tert-butoxycarbonyl)-L-lysinyl succinimidate (200 mg, 0.298 mmol, 2.0 eq.) in THF (8 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer extracted, washed with water (2 x 10 mL) and brine (20 mL). After drying (Na_2SO_4), filtration and evaporation, the residue was purified on SiO_2 (eluent : $CHCl_3$ / MeOH / NEt_3: 91 / 8 / 1, Rf = 0.30). Et_2O is then added and the resulting white solid filtered off. Yield : 0.112 g (0.076 mmol, **36 %**). ^1H NMR (400 MHz, d_6-DMSO) : δ 7.75 (m, 6 H, 2 x 2 NH^{α} and 2 NHC^{1}), 7.60 (d, 2 H, J = 8.0, 2 NH^{α}), 6.85 (m, 2 H, 2 NH), 6.68 (m, 4 H, 2 NH), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.10 (m, 4 H, 2 x 2 CH^{α}), 3.80 (m, 2 H, 2 CH^{α}), 3.00 (m, 6 H, 2 CH_2^{ε} and 2 $CH^{1'}$), 2.75 (m, 2 H, 2 $CH^{1'}$), 2.84 (m, 8 H, 2 x 2 CH_2^{ε}), 2.30 (m, 8 H, 4 $CH_2^{4'}$), 2.19 (m, 4 H, 2 $CH_2^{3'}$), 2.06 (t, 4 H, J = 9.0, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.65 – 1.38 (m, 18 H), 1.32 (s, 54 H, 2 x 3 $C(CH_3)_3$), 1.30 – 1.15 (m, 48 H), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 8

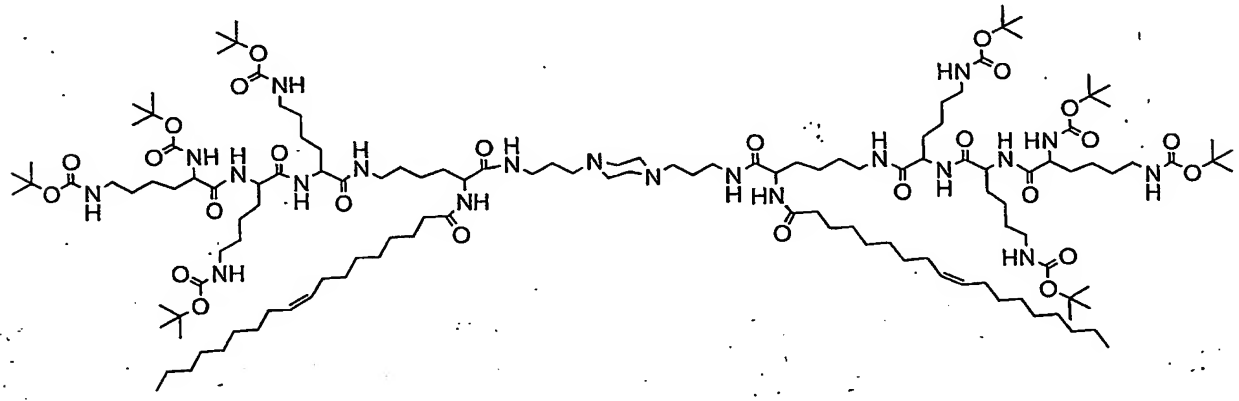
RG 00/804



To a solution of RG00/794 (110 mg, 0.052 mmol) in MeOH (7 mL) was added concentrated HCl (7 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vaccuum using EtOH to coevaporate. The residue was dissolved in water, filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected as a white powder. Yield : 88 mg (0.049 mmol, 94 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.69 (d, 2 H, J = 7.8, 2 $NH^{\alpha 2}$), 8.30 (m, 2 H, 2 $NH^{\alpha 1}$), 8.12 (m, 2 H, 2 NHC^{1}), 7.95 (m, 12 H, 2 $NH^{\varepsilon 2}$, 2 $NH^{\varepsilon 3}$ and $NH^{\alpha 3}$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.20 (m, 2 H, 2 $CH^{\alpha 1}$), 4.08 (m, 2 H, 2 $CH^{\alpha 3}$), 3.84 (m, 2 H, 2 $CH^{\alpha 2}$), 3.65 (m, 2 H, 2 NH^{+}), 3.10 (m, 12 H, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 3.05 (m, 2 H, 2 $CH^{1'}$), 2.95 (m, 2 H, 2 $CH^{1'}$), 2.74 (m, 8 H, 2 $CH_2^{\varepsilon 1}$ and 2 $CH_2^{\varepsilon 2}$), 2.11 (t, 4 H, J = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.80 – 1.12 (m, 82 H), 0.84 (t, 6 H, J = 6.4, 2 CH_3^{18}). MS (+ES) : m/z $[M+H]^{2+}$ 750.1.

Example 9

RG 00/797

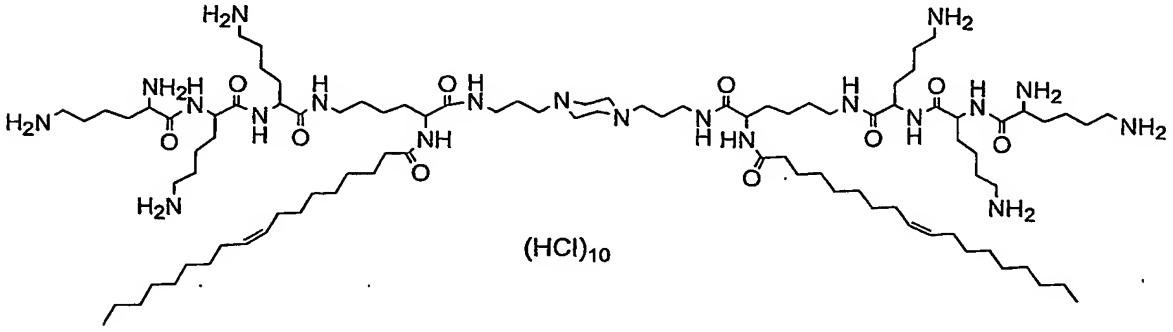


To a solution of bis aminocompound (140 mg, 0.142 mmol) in THF (48 mL) was added successively a solution of K_2CO_3 (40 mg, mmol, 2.1 eq.) in water (10 mL) and $(Boc)_4$-KKKOSu (256 mg, 0.284 mmol, 2.0 eq.) in THF (10 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer was extracted, washed with 5 % K_2CO_3, water (10 mL) and brine (20 mL). After drying (Na_2SO_4), filtration and evaporation, the residue was purified on SiO_2 (eluent : $CHCl_3$ / MeOH / NEt_3: 85 / 15 / 1, Rf = 0.32). Et_2O is then added and the resulting white solid filtered off. Yield : 0.310 g (0.121 mmol, 85 %). ^{1}H NMR (400 MHz, d_6-DMSO) : δ 8.00 (m, 2 H, 2 NH^{α}), 7.85 (m, 2 H, 2 NH^{α}), 7.75

(m, 4 H, 2 NH^{α} and 2 NHC^{1}), 6.85 (m, 2 H, 2 $N^{\alpha}H$), 6.68 (m, 6 H, 2 x 3 $N^{\varepsilon}H$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.18 (m, 2 H, 2 CH^{α}), 4.09 (m, 4 H, 2 x 2 CH^{α}), 3.82 (m, 4 H, 2 x 2 CH^{α}), 3.00 (m, 6 H, 2 CH_2^{ε} and 2 $CH^{1'}$), 2.75 (m, 2 H, 2 $CH^{1'}$), 2.84 (m, 12 H, 2 x 3 CH_2^{ε}), 2.47 (m, 8 H, 2 x 2 $CH_2^{4'}$), 2.29 (m, 4 H, 2 $CH_2^{3'}$), 2.09 (t, 4 H, J = 9.0, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.65 – 1.15 (m, 168 H), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 10

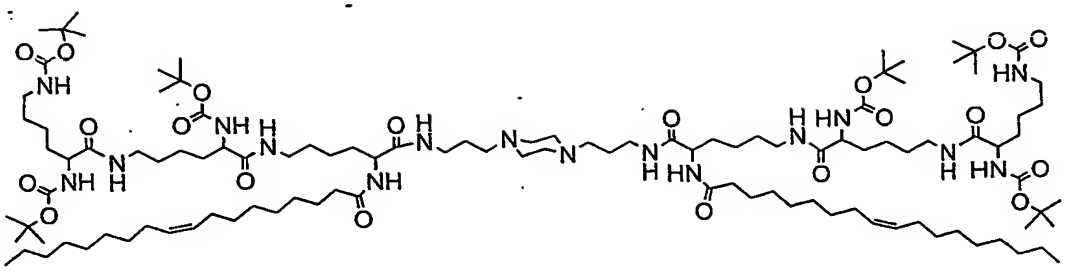
RG 00/805



To a solution of RG00/797 (110 mg, 0.052 mmol) in MeOH (7 mL) was added concentrated HCl (7 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vaccuum using EtOH to coevaporate. The residue was dissolved in water (40 mL), filtered and evaporated. The compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected as a pale brown powder. Yield : 88 mg (0.049 mmol, 94 %). ^1H NMR (400 MHz, d_6-DMSO) : δ 8.80 (d, 2 H, J = 7.8, 2 NH^{α}), 8.30 (m, 6 H, 2 x 3 NH^{α}), 8.03 (m, 14 H, 2 NHC^{1} and 2 x 3 $N^{\varepsilon}H_3^{+}$), 5.30 (m, 4 H, 2 $CH^{9,10}$), 4.28 (m, 2 H, 2 CH^{α}), 4.18 (m, 2 H, 2 CH^{α}), 4.08 (m, 2 H, 2 CH^{α}), 3.85 (m, 2 H, 2 CH^{α}), 3.65 (m, 2 H, 2 NH^{+}), 3.10 (m, 16 H, 2 $CH_2^{\varepsilon 1}$, 2 $CH_2^{3'}$ and 2 x 2 $CH_2^{4'}$), 3.02 (m, 2 H, 2 $CH^{1'}$), 2.95 (m, 2 H, 2 $CH^{1'}$), 2.74 (m, 8 H, 2 x 3 CH_2^{ε}), 2.10 (t, 4 H, J = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.71 (m, 4 H, 2 CH_2^{β}), 1.60 – 1.17 (m, 108 H), 0.84 (t, 6 H, J = 6.4, 2 CH_3^{18}).. MS (+ES) : m/z $[M+H]^{2+}$ 750.1.

Example 11

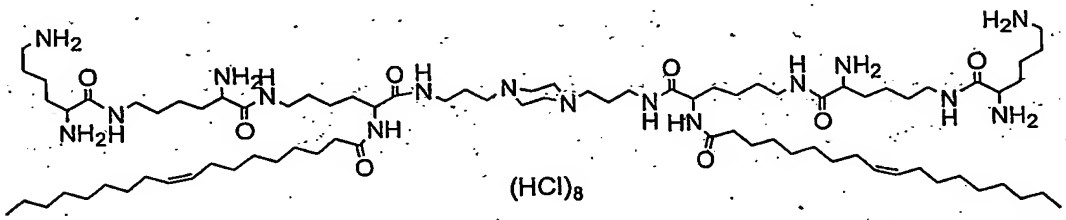
RG 00/823



To a solution of bis aminocompound (76 mg, 0.077 mmol) in THF (40 mL) was added successively a solution of K_2CO_3 (22 mg, 0.159 mmol, 2.06 eq.) in water (2 mL) and Boc_3(K-ε-K)-OSu (105 mg, 0.156 mmol, 2.0 eq.) in THF (8 mL). The reaction was then stirred for 16 h at RT. Most of THF was evaporated and the residue redissolved in $CHCl_3$. Water (10 mL) was added and the organic layer extracted, washed with water (2 x 10 mL) and brine (20 mL). After drying (Na_2SO_4), filtration and evaporation, the residue was purified on SiO_2 (eluent : $CHCl_3$ / MeOH / NEt_3: 91 / 8 / 1, Rf = 0.30). Et_2O is then added and the resulting white solid filtered off. Yield : 0.124 g (0.059 mmol, 77 %). ^1H NMR (400 MHz, d_6-DMSO) : δ 7.79 (m, 4 H, 2 NH^{α} and 2 NH^{ε}), 7.67 (m, 4 H, 2 NHC^{1} and 2 NH^{ε}), 6.69 (m, 2 H, 2 NH^{ε}), 8.28 (m, 8 H, 2 x 2 NH^{α}), 5.28 (m, 4 H, 2 $CH^{9,10}$), 4.10 (m, 2 H, 2 $CH^{\alpha 1}$), 3.78 (m, 4 H, 2 x 2 CH^{α}), 3.00 (m, 6 H, 2 CH^{ε} and 2 $CH^{1'}$), 2.98 (m, 2 H, 2 $CH^{1'}$), 3.13 (m, 12 H, 2 x 2 CH_2^{ε}), 2.47 (m, 4 H, 2 $CH_2^{3'}$), 2.25 (m, 8 H, 2 x 2 $CH_2^{4'}$), 2.08 (t, 4 H, J = 7.2, 2 CH_2^{2}), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.80 – 1.14 (m, 138 H), 0.82 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 12

RG 00/830



To a solution of RG00/823 (124 mg, 0.059 mmol) in MeOH (10 mL) was added concentrated HCl (6 mL). The reaction was stirred at RT for 1 h and the solvent were then removed under vaccuum using EtOH to coevaporate. The residue was dissolved in water (40 mL), filtered and evaporated. The

compound was dissolved in a minimum amount of MeOH and precipitated with Et_2O. The resulting solid was filtered and collected as a pale pink powder. Yield : 101 mg (0.056 mmol, 96 %). ^1H NMR (400 MHz, d_6-DMSO) : δ 8.73 (t, 2 H, J = 7.8, 2 NH^{ε}), 8.67 (m, 2 H, 2 $NH^{\varepsilon 1}$), 8.28 (m, 8 H, 4 NH_2^{α}), 8.15 (m, 6 H, 2 NHC^1, 2 $NH^{\varepsilon 2}$ and 2 $NH^{\varepsilon 3}$), 7.99 (m, 2 H, 2 $NH^{\alpha 1}$), 5.29 (m, 4 H, 2 $CH^{9,10}$), 4.09 (m, 2 H, 2 $CH^{\alpha 1}$), 3.72 (m, 4 H, 2 $CH^{\alpha 3}$ and 2 $CH^{\alpha 2}$), 3.65 (m, 2 H, 2 NH^{+}), 3.08 (m, 10 H, 2 $CH_2^{\varepsilon 1}$, 2 $CH_2^{\varepsilon 2}$, 2 $CH_2^{3'}$ and 2 $CH_2^{4'}$), 2.74 (m, 2 H, 2 $CH_2^{1'}$), 2.11 (t, 4 H, J = 7.2, 2 CH_2^2), 1.95 (m, 8 H, 2 $CH_2^{8,10}$), 1.80 – 1.14 (m, 82 H), 0.83 (t, 6 H, J = 6.4, 2 CH_3^{18}).

Example 13

Delivery of fluorescent oligonucleotides to cell lines/primary cells using Gemini Surfactant 170 (GS170)

GS170 (1 mg/ml in water) was diluted to a 10x solution with Optimem serum free media. A FITC-tagged oligonucleotide was similarly diluted in Optimem at 10x final concentration. The GS170 and oligonucleotide were then mixed 1:1 and incubated for fifteen minutes at room temperature. The adherent cell lines: RBL-2H3, J774 and 16HBE14o were plated out the day before transfection. Murine primary T cells were transfected either inactivated or after differentiation into T helper 2 cells.

GS170:oligo complexes were diluted to 1x in Optimem and added to adherent cells that had been washed once in Optimem then all media removed. Nuclear delivery of the oligonucleotide was oserved over a period of 24 hours and compared to the commercial reagent, Lipofectamine 2000 (LF2K).

Optimal transfection efficiencies were as follows:

	GS170 (%nuclear)	LF2K (%nuclear)
RBL-2H3	**50%**	**#**
J774	**50%**	**50%**
16HBE14o	**50%**	**30%**
Primary inactivated T cells	**60%**	**#**
Activated T helper 2 cells	**60%**	**#**

 # = too low to estimate